

Long-Term Carcinogenicity Study in Syrian Golden Hamster of Particulate Emissions From Coal- and Oil-Fired Power Plants

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Male Syrian golden hamsters were given 15 weekly intratracheal instillations with suspensions of coal fly ash or oil fly ash. Controls were instilled with saline containing gelatine (0.5 g/100 mL) or to check particle effects with suspensions of hematite (Fe_2O_3). The common weekly dose was 4.5 mg/hamster. In addition, one subgroup of hamsters was treated with oil fly ash at a weekly dose of 3.0 mg/hamster and another with coal fly ash at a weekly dose of 6.0 mg/hamster. Other groups of hamsters were treated with suspensions of benzo[a]pyrene (BaP) or with suspensions on coal fly ash, oil fly ash, or Fe_2O_3 coated with BaP. The mass median aerodynamic diameters of the coal and oil fly ashes were 4.4 μm and 28 μm , respectively. Hamsters treated with oil fly ash showed a higher frequency of bronchiolar-alveolar hyperplasia than hamsters in the other treatment groups. Squamous dysplasia and squamous metaplasia were most frequent in animals treated with suspensions of BaP or BaP-coated particles. The earliest appearance of a tumor, the highest incidence of tumors, and the highest incidence of malignant tumors were observed in hamsters treated with oil fly ash coated with BaP. Squamous cell carcinoma and adenosquamous carcinoma were the most frequent malignant tumors. No malignant tumors and only few benign tumors were observed in hamsters instilled with suspensions of fly ash not coated with BaP. The present study gives no indication that coal fly ash could create more serious health problems than oil fly ash.

Introduction

Sweden has the intention to reduce its heavy dependence on oil and, in the future, also reduce its dependence on nuclear power as sources of energy. To compensate for this loss of energy production, there are plans to reintroduce coal as an energy source. Today the usage of coal for production of heat and electricity is negligible. For this reason the Swedish government initiated a research program, the Coal-Health-Environment Project, on various aspects of increased coal utilization (1). The present long-term carcinogenicity study is a part of this program. The main emphasis was to study the carcinogenic effects of coal fly ash, but an attempt to compare with oil fly ash was also made. The fly ash particles examined in the present investigation were sampled in a manner to be, as far as possible, representative of oil and coal fly ash particles emitted into the environment (2). The particulate material has also been characterized physically, chemically, and, in

part, biologically (2,3). The chemical analyses showed that the sampled materials contained very low amounts of polycyclic aromatic hydrocarbons (PAH), suggesting an effective combustion in the power plants during sampling (2). However, under less favorable conditions, more PAH could be emitted. We therefore coated part of the material with a PAH, benzo[a]pyrene (BaP). As the respiratory pathways were considered to be the most interesting region in our carcinogenicity study, an administration of the experimental material by inhalation would have been preferable. However, the small amount of sampled materials and our insufficient facilities for performing a large-scale inhalation study in a great number of animals made us choose another mode of administration in the present investigation, intratracheal instillation. Intratracheal instillation in Syrian golden hamsters of particulate material coated with BaP has been shown by Saffiotti et al. (4-6) and recently by Pershagen et al. (7) to be a useful model to study carcinogenicity in the respiratory pathways.

Long-term studies in several species on the carcinogenicity of coal fly ash have been reported (8-10). No studies have shown that coal fly ash is carcinogenic. As far as we know, hitherto, no long-term investigation on the carcinogenicity of particulate emissions from an oil-fired power plant has been presented. This is also the

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first study in which a comparison is made between the carcinogenic effects of particulate emissions from a coal-fired and an oil-fired power plant.

Materials and Methods

Sampling, Characterization, and Preparation of the Particulate Material

Sampling, characterization, and preparation of the particles used in the tests have been described in detail elsewhere (2,3). The following is a summary.

Sampling. To get samples that were similar to the emissions from the stacks, a dilution and cooling probe was used in which the flue gas was cooled by dilution with filtered ambient air in a ratio of 1:10. After passing the probe, the particles were collected on stainless-steel grids in a Sierra High Volume impactor. Particles, which passed the final stage of the impactor (cut size 0.5 μm aerodynamic diameter), were collected on a Gelman glass fiber filter. Periodically the sampling was stopped, and the particulate material collected on the impactor stages was scraped off with a Teflon spatula. Samples of flue gas particles were drawn isokinetically from the stack before and after the electrostatic precipitator for the oil-fired and coal-fired power plant, respectively. The sampling method used made it impossible to collect the amount of oil fly ash needed for the study after the electrostatic precipitator. The concentration at this sampling point was too low.

Characterization. The morphology of the fly ash particles from the impactor stages and the glass fiber filters was studied with electron and light microscopy. The coal fly ash particles were solid spheres, whereas the oil fly ash particles were hollow scenospheres with a porous structure. Particle size distributions of the size fractions suspended in 0.9% saline were determined with a Coulter Counter TA II. The measured volume median diameters corresponded to those expected from the cut sizes of the impactor, except for the filter fractions, which showed larger sizes. This was especially evident for the oil fly ash, indicating bounce off in the impactor during sampling. The fly ashes for use in the biological tests were then prepared by blending the different size fractions in proportions corresponding to their average percent by weight of the total fly ash material collected. Since it was not possible to avoid contamination of glass fibers in the filter fraction of the coal fly ash, this fraction was omitted in the blending of the coal fly ash material (2). The size distribution, density, and specific surface area of the blended fly ashes used in the biological tests were then determined. The resulting volume median diameters (VMD) and geometric standard deviations, σ_g , are given in Table 1. The density of the fly ash particles was determined with a gas pycnometer and by liquid displacement with the particles in xylene and ethanol. For the coal fly ash and the oil fly ash, the densities were 2.3 g/cm³ and 2.0 g/cm³, respectively. Specific surface area, determined

with nitrogen adsorption (BET method), was 4.0 m²/g for the coal fly ash and 2.3 m²/g for the oil fly ash.

From the morphology, density, and volume median diameter, the mass median aerodynamic diameter (MMAD) for the coal fly ash particles was calculated. The result is given in Table 1. Because the oil fly ash particles showed a porous hollow structure, their MMAD could not be directly calculated from VMD and density. For that reason the particle size distribution of these particles was also determined with a sedimentation instrument (Sedigraph 5000), giving a Stoke's diameter of 20 μm and a geometric standard deviation, σ_g , of 1.7. Corresponding MMAD for the oil fly ash is given in Table 1.

The inorganic chemical composition of the size fractions and the blended fly ash material was determined with Proton Induced X-ray Emission spectroscopy (PIXE). The results of these analyses are published elsewhere (2,3). Results of specific interest for the blended materials were, for coal fly ash: S, 1.2%; V, 800 ppm; Ni, 240 ppm; Cu, 260 ppm; Zn, 990 ppm; As, 65 ppm; Se, 29 ppm; Pb, 510 ppm; Th, 32 ppm; and for oil fly ash: S, 10.4%; V, 1.2%; Ni, 3200 ppm; Cu, 110 ppm; Zn, 340 ppm; As, < 5 ppm; Se, 9 ppm; Pb, 90 ppm; and Th, < 15 ppm.

The blended fly ashes were also analyzed for the organic component benzo[a]pyrene. In both the coal and the oil fly ash, BaP was below detection limit (< 1 ppm, using HPLC and fluorescence detector).

Benzo[a]pyrene coating. Benzo[a]pyrene-coated particles were prepared with the technique described by Saffiotti et al. (5). Equal amounts of BaP (~ 98%, Sigma, St. Louis, MO), as a 10% solution in acetone, and particulate material, as a 0.2% suspension in cold (4°C) water, were mixed. Upon mixing, the BaP crystallized onto the particles, which were then filtered off on a membrane filter (0.4 μm Nuclepore). Pure BaP particles were prepared in the same way without particles in the cold water. Before instillation, the particulate material was scraped off the filter and resuspended in 0.9% saline solution by ultrasonication. The volume median diameter of the resuspended particles was determined with Coulter Counter. The results are shown in Table 1. The fact that the geometric standard deviation is about the same and that the volume median di-

Table 1. Physical parameters showing the size of the various particles.

Particulate material	VMD, μm^a	σ_g^b	MMAD, μm^c
Coal fly ash	2.9	1.9	4.4 \pm 0.2
Oil fly ash	17	2.0	28 \pm 1.2
Fe ₂ O ₃	< 1	—	
BaP	3.7	1.6	
Coal fly ash + BaP	3.3	2.0	
Oil fly ash + BaP	17	2.0	
Fe ₂ O ₃ + BaP	1.8	1.6	

^aVMD, volume median diameter for the material.

^b σ_g , geometric standard deviation.

^cMMAD, calculated mass median aerodynamic diameter given as mean \pm 1 SD.

ameter is somewhat larger for the BaP-coated particles when compared with the corresponding uncoated particles indicates that the BaP is associated with the particles. This is especially evident for the oil fly ash. Microscopic studies of the BaP-coated particles, using fluorescent UV light, also confirms that the BaP is coated onto the particles. This is in agreement with the findings of Saffiotti (5), who used the same coating procedure.

Animals

Outbred male Syrian golden hamsters, quality 2 (Bantin and Kingman, Hull, Great Britain) were used. The hamsters were housed individually, and the cages were placed in two identical rooms. In one room, the animals treated with benzo[a]pyrene-coated material were housed, and in the other room, hamsters treated with material not coated with benzo[a]pyrene were housed. The room temperature was 21 to 24°C. The relative humidity was $50 \pm 5\%$. Artificial light was the only source of light available. The hamsters were set on a 12 hr day and night light cycle, with light on at 6 A.M. and off at 6 P.M. The animals were fed Ewos commercial diet R3 in pellets and tap water *ad libitum*. During the 15 weeks of treatment the hamsters were weighed once a week. Thereafter, their body weights were recorded every 2 to 3 weeks. Each animal was examined daily and observations on their health were recorded. Before treatment the hamsters were allowed to acclimatize for 2 to 3 weeks. At the beginning of the experiments the animals were 8- to 13-weeks old and weighed about 80 to 90 g. Animals were selected for the various treatments by a randomization procedure.

Treatment

The particulate material, prepared as described above, was instilled intratracheally. The technique used for intratracheal instillation was a modification of the method described by Saffiotti et al. (5). The hamsters were anesthetized with methohexital (Brietal, Eli Lilly and Company, Indianapolis, IN) 40 to 50 mg/kg IP. The anesthetized animal was placed in a special apparatus

that allowed proper fixation during the treatment. For instillation, we used a device made of Teflon and consisting of a cylinder with a conical end fitted with a thin (0.75 mm), 100-mm long, stainless-steel tube, bent at an angle of about 135°. The device was attached to a 1-mL disposable syringe. Before use, the device was sterilized in an autoclave. The intratracheal instillation was performed in the following way. Under careful inspection, the tip of the tube was put approximately 5 mm down into the larynx of the anesthetized hamster without touching the inner laryngeal walls. The material, consisting of a 0.2 mL particulate suspension in sterile saline with 0.5% gelatine added, was delivered. In order to obtain a homogeneous suspension, it was necessary to omit the gelatine in the suspensions made of uncoated oil fly ash. A detailed description of the preparation of the suspensions is found above. After checking the proper delivery of the suspension to the hamster, the animal was released from the instillation equipment and allowed to wake up from the anesthesia. The hamster was carefully watched under this procedure. Frequently, an animal showed apnea, and sometimes it was necessary to help it breathe by gentle massage of the thorax. The different groups of treatment, as well as the control groups, are shown in Table 2. Each hamster was instilled once a week for 15 weeks. The animals not surviving three instillations were replaced. For practical reasons, we could not instill all hamsters at the same time, therefore, the animals were divided into three subgroups. The number of animals in these groups were 140, 204, and 186. All treatments, as well as the corresponding controls, were represented in the different groups and in a similar frequency for each group. The experimental design of the study has been approved by the Regional Research Ethical Committee according to national laws (SFS 1970:286, SFS 1982:554).

All animals with clinical symptoms so severe that the hamsters could be characterized as moribund were sacrificed by injection of methohexital (see below). The basis for this sacrifice was mostly ethical, i.e., to decrease the suffering of the moribund hamsters. We planned to terminate the study 150 weeks after beginning the treatment. No hamsters survived at that time.

Table 2. Treatment of hamsters.

Code	Treatment	Single dose levels, mg/hamster	Number of animals (test design)	Number of animals (final) ^a
A	Sterile saline + gelatine (0.5 g/100 mL), vehicle control	—	60	60
Ba	Coal fly ash (low dose)	4.5	60	58
Bb	Coal fly ash (high dose)	6.0	30	30
Ca	Oil fly ash (high dose)	4.5	60	59
Cb	Oil fly ash (low dose)	3.0	30	29
D	Ferric oxide (Fe ₂ O ₃), particle control	4.5	60	58
E	Sterile saline + gelatine (0.5 g/100 mL) + BaP	4.5	60	59
F	Coal fly ash + BaP	4.5 + 4.5	60	59
G	Oil fly ash + BaP	4.5 + 4.5	60	58
H	Fe ₂ O ₃ + BaP	4.5 + 4.5	60	60
Total			540	530

^aAll hamsters that died before three instillations were performed were replaced by new animals.

Administered Dose

The doses of the various particles in 0.2 mL of sterile saline or saline with gelatine (0.5 g/100 mL) were selected after consideration of the results of the acute and subacute toxicity studies reported elsewhere (2) and of data reported in earlier investigations (6,7,11,12), in which suspensions of particles had been instilled intratracheally. As the particle control we used hematite, ferric oxide Fe_2O_3 (Fischer Scientific Company, Fair Lawn, NJ). To ease comparisons between the groups, we gave all hamsters the same dose (4.5 mg/hamster), except in two subgroups. Since oil fly ash was found to be more toxic than the other examined particles, we decided to have a subgroup of hamsters with a lower dose of oil fly ash (3 mg/hamster). Coal fly ash had low toxicity; therefore, we instilled a subgroup of hamsters with a higher dose (6 mg/hamster). Hamsters instilled with suspensions of particles coated with BaP were given a single dose of 9 mg/hamster. The actual dose delivered by the instillation device was tested by instillations in test tubes. Instillations with uncoated particles showed a satisfactory reproducibility. In each instillation, 85 to 90% of accurately weighed fly ash and ferric oxide was delivered. Instillations of BaP and BaP-coated particles showed less reproducibility, and only about 50% by weight of material was delivered in each instillation. For this reason it was decided to make parallel instillations in test tubes with BaP and BaP-coated particles at each weekly treatment. The test tubes were then analyzed for BaP by gas chromatography. The results are shown in Table 3. From these data, the cumulative doses of BaP in the lung was estimated to be 33 ± 9 mg (BaP), 32 ± 11 mg (coal fly ash + BaP), 24 ± 8 mg (oil fly ash + BaP), and 30 ± 9 mg (Fe_2O_3 + BaP).

In another study, the retention of BaP in the lung after a single instillation of BaP and BaP-coated particles was investigated (2). These results are also shown in Table 3. We have also made some additional studies on the distribution of BaP in kidney, liver, and gastrointestinal tract (GI) various times after a single instillation of BaP and particles coated with BaP (13). The concentrations of BaP in the GI was 300 to 1000 times lower than the initial (0.5 hr) lung concentration.

Morphological Examinations

All animals, which had been instilled 15 times, that had died either spontaneously or moribund hamsters

killed by methohexital (100 mg/kg IP) were autopsied. Autopsy of a dead hamster was always performed within 24 hr after death. In order to avoid collapse of the lungs and to fix them expanded, the lungs were removed as described by Saffiotti et al. (5). Macroscopical findings at autopsy were recorded. The lungs and other tissues were fixed in 10% formaldehyde in a phosphate buffer 0.1 M, pH 7.4. Paraffin sections at various levels of larynx (transverse sections), trachea (longitudinal sections), and all lung lobes were stained with hematoxyline-eosine. The sections were examined microscopically by a pathologist, who had no knowledge about the treatment of the hamster. The pathological lesions were evaluated mainly according to the criteria used by Saffiotti et al. (5,6) and Stenbäck (14). All malignant tumors and those benign tumors that were histologically benign, but fatal due to their location or largeness, were considered as fatal tumors.

Statistical Analysis

Survival rates were determined according to Elandt-Johnson and Johnson (15). Pairwise comparisons between curves were performed according to Mantel and Haenzel in Elandt and Johnson (15). Comparison between three curves concerning a positive trend were done according to Peto et al. (16). The probability of the tumor incidence was determined as described by Saffiotti et al. (6) according to the formula

$$P_n = 1 - \frac{(N_1 - t_1)}{N_1} \times \frac{(N_2 - t_2)}{N_2} \dots \times \frac{(N_i - t_i)}{N_i} \dots \times \frac{(N_n - t_n)}{N_n}$$

P_n = Probability to die with a tumor in week n

N_n = Number of survivals in the beginning of week n

t_n = Number of hamsters that die with tumor during week n

The testing of the significance of the differences in tumor incidence between various groups were performed according to Peto (17) and Peto et al. (18). By these calculations, hamsters that died from tumor(s) were separated from hamsters that died from other causes, but had a tumor at autopsy.

Table 3. Delivered dose and retention of BaP in the lung of the hamster.

Code	Treatment	Single dose, mg (particle + BaP) ^a	Delivered dose, mg BaP ^a	% BaP in the lung after a single instillation at ^b			
				0.5 hr	3 hr	24 hr	168 hr
E	BaP	0 + 4.5	2.2 ± 0.6	75	20	4	3.5
F	Coal fly ash + BaP	4.5 + 4.5	2.1 ± 0.7	70	28	2	1
G	Oil fly ash + BaP	4.5 + 4.5	1.6 ± 0.5	80	38	21	5
H	Fe_2O_3 + BaP	4.5 + 4.5	2.0 ± 0.6	90	40	1	0.5

^aEach value represents the mean \pm 1 SD of four determinations.

^bMean of four animals.

Results

Body Weight

The body weight gives a measure of the general health of the experimental animals. Figure 1 shows the changes in the mean weight of the various groups of hamsters. Within 8 to 15 weeks after beginning the treatment, a weight gain was observed in all groups except in groups treated with oil fly ash. In these groups there was a loss of weight. Decreased body weight was seen in all groups 65 to 70 weeks after start of the treatment. In hamsters treated with BaP or with BaP-coated particles (except with oil fly ash), the body weight did not change between 15 to 40 weeks after beginning the treatment, but decreased after 40 to 45 weeks. The earliest onset and the most pronounced decrease in body weight was found in the group treated with the high dose of oil fly ash and in the group treated with oil fly ash coated with BaP. There was no difference in change of body weight between these two groups.

Survival Rates

Figure 2 shows the survival rates (percent of the initial number of animals) of the hamsters in experimental and control groups. Hamsters treated with oil fly ash (high dose) and oil fly ash coated with BaP had a significantly lower survival rate than their respective controls. However, the two groups of hamsters intratracheally instilled with oil fly ash (high dose and low dose) did not significantly differ from those instilled with ferric oxide, the particle controls. The hamsters in the

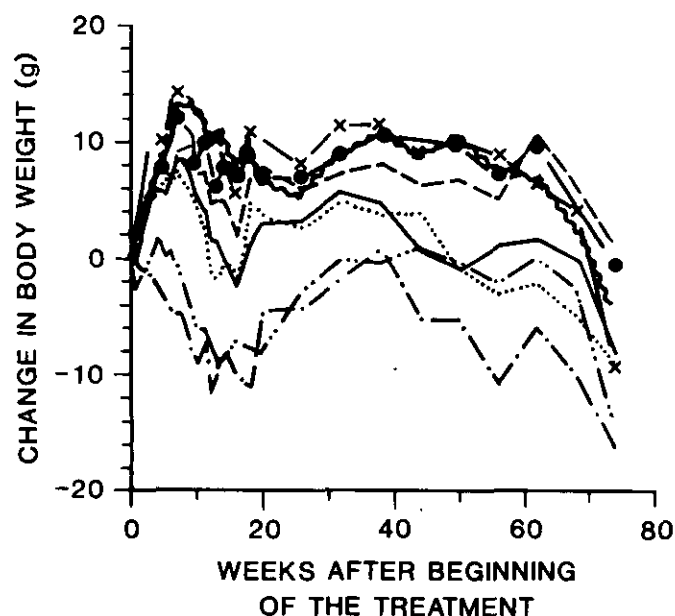


FIGURE 1. Changes in the mean weight of the various groups of hamster. Saline (x—x); coal fly ash (low dose)(~); oil fly ash (high dose) (---); Fe_2O_3 (●—●); BaP (—); coal fly ash coated with BaP (---); oil fly ash coated with BaP (---); Fe_2O_3 coated with BaP (—).

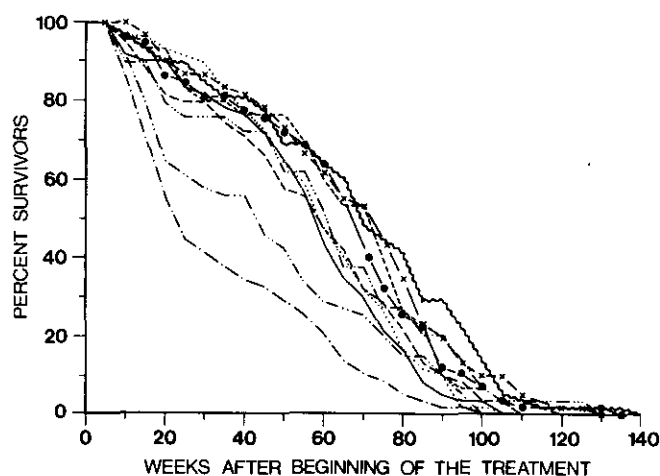


FIGURE 2. Survival rates (percent of the initial number of animals). Saline (x—x); coal fly ash (low dose)(~); coal fly ash (high dose) (---); oil fly ash (high dose) (---); oil fly ash (low dose) (---); Fe_2O_3 (●—●); BaP (—); coal fly ash coated with BaP (---); oil fly ash coated with BaP (---); Fe_2O_3 coated with BaP (—).

various groups treated with oil fly ash also had a significantly lower survival rate than hamsters treated with coal fly ash or coal fly ash coated with BaP. All groups of hamsters instilled with coal fly ash had a survival rate that did not differ from the corresponding controls.

Morphological Findings

Epithelial Changes Not Classified As Tumors.

Varying degrees of changes in the epithelium of larynx, trachea, and bronchi-bronchioli were observed (Table 4). In the larynx, squamous epithelial hyperplasia was most frequent in hamsters treated with BaP and occurred in high frequency also in hamsters treated with oil fly ash coated with BaP and ferric oxide coated with BaP. However, the fairly high frequency of laryngeal squamous epithelial hyperplasia in hamsters treated with saline or with ferric oxide suggests that this lesion in the larynx could be a reaction to intratracheal instillation *per se* rather than to the particulate material.

Hamsters treated with BaP or BaP-coated particles had a higher frequency of squamous metaplasia than those given suspensions without BaP in trachea but not in the bronchi. Also, a higher frequency of squamous dysplasia was found in the larynx and trachea but not in the bronchi of hamsters treated with BaP or BaP-coated particles.

Bronchiolar-alveolar hyperplasia was not observed in hamsters treated with saline. However, reaction of low intensity was observed in several hamsters treated with either ferric oxide, oil fly ash (low dose), and coal fly ash (low dose). No significant changes were observed between controls and the various treatments or between the various treatments. When all hamsters with more pronounced bronchiolar-alveolar hyperplasia were examined, it was found that animals treated with oil fly

Table 4. Epithelial changes not classified as tumors.

Sites and types of lesion	Incidences of lesions									
	Group A	Group Ba	Group Bb	Group Ca	Group Cb	Group D	Group E	Group F	Group G	Group H
Squamous epithelial hyperplasia of the larynx	9/60 (1,0)*	10/58 (1,4)	7/30 (1,1)	7/59 (1,1)	5/29 (1,4)	12/58 (1,0)	24/59 (1,1)	12/59 (1,3)	17/58 (1,4)	24/60 (1,2)
Squamous metaplasia of the trachea	9/60 (1,0)	4/58 (1,0)	5/30 (1,0)	15/59 (1,1)	12/29 (1,0)	9/58 (1,0)	24/59 (1,1)	10/59 (1,2)	20/58 (1,4)	18/60 (1,1)
Squamous metaplasia of the bronchi	1/60 (—)	1/58 (—)	2/30 (—)	4/59 (1,0)	1/29 (—)	3/58 (1,0)	3/59 (1,0)	3/59 (1,0)	11/58 (1,0)	6/60 (1,0)
Squamous cell dysplasia of the larynx	11/60 (1,1)	13/58 (1,1)	3/30 (1,0)	2/59 (—)	6/29 (1,3)	9/58 (1,1)	18/59 (1,4)	13/59 (1,2)	18/58 (1,6)	15/60 (1,4)
Squamous cell dysplasia of the trachea	2/60 (—)	1/58 (—)	1/30 (—)	0/59	0/29	0/58	8/59 (1,3)	1/59 (—)	8/58 (1,4)	1/60 (—)
Squamous cell dysplasia of the bronchi	0/60	2/58 (—)	1/30 (—)	0/59	1/29 (—)	1/58 (—)	1/59 (—)	0/59	8/58 (1,0)	0/60
Bronchiolar-alveolar hyperplasia	0/60	4/58 (1,0)	2/30 (—)	26/59 (1,7)	11/29 (1,3)	7/58 (1,0)	6/59 (1,2)	7/59 (1,1)	25/58 (2,1)	4/60 (1,3)

*Fraction represents frequency of occurrence. Numbers in parentheses are average degree of severity.

ash (high dose) had a significantly higher frequency of lesions than the animals treated with coal fly ash and also a higher frequency than the vehicle and particle control groups. A similar result was also obtained when the various groups including all hamsters with bronchiolar-alveolar hyperplasia of any degree were compared. We also found a significantly higher frequency of bronchiolar-alveolar hyperplasia in animals treated with oil fly ash coated with BaP than in hamsters treated with coal fly ash coated with BaP.

Tumor Incidence. Hamsters instilled with suspensions containing BaP had the highest tumor incidence. Furthermore, all fatal benign (i.e., histologically benign tumor that is fatal due to its location or largeness) and all malignant tumors were found in these hamsters. Among 58 animals treated with oil fly ash coated with BaP, there were 18 hamsters (31%) with malignant tumors, whereas only 2 animals (3%) in the group treated

with coal fly ash coated with BaP had malignant tumors. It is noticeable that animals treated with BaP alone had a higher incidence of malignant tumors than those instilled with ferric oxide coated with BaP. In hamsters given oil fly ash coated with BaP, the malignant tumors occurred earlier than in the other groups. The first malignant tumor in this group was found already 16 weeks after beginning the instillations. Malignant lymphoma affecting the lungs were observed in two animals. Such tumors were found in one hamster treated with ferric oxide and in another treated with oil fly ash (low dose). Malignant lymphoma occurs spontaneously in the Syrian golden hamster (19). The frequency and distribution of various types of tumors are shown in Table 5. All but three squamous cell papillomas observed were in the larynx and trachea. The highest frequency of papilloma was found in hamsters treated with suspensions containing BaP. A few papillomas were large enough to

Table 5. Number and type of tumors in various regions of the respiratory tract.*

Code	Treatment	N	Squamous cell papilloma				Squamous cell carcinoma			Adenosquamous carcinoma		
			Larynx	Trachea	Bronchi	Lungs	Larynx	Trachea	Lungs	Larynx	Trachea	Lungs
A	Vehicle	60	1									
Ba	Coal fly ash (low dose)	58	1									
Bb	Coal fly ash (high dose)	30										
Ca	Oil fly ash (high dose)	59		1								
Cb	Oil fly ash (low dose)	29										
D	Ferric oxide, Fe ₂ O ₃	58	1									
E	BaP	59	3	4			2	1	3			2
F	Coal fly ash + BaP	59	9						2			
G	Oil fly ash + BaP	58	2	8	3		6 ^b	5 ^b	3	1 ^b	3 ^b	6
H	Fe ₂ O ₃ + BaP	60	8	1			2	2			1	

*Two adenomas were observed. One adenoma was in a hamster treated with the high dose oil fly ash and one was in a hamster treated with oil fly ash + BaP. Two adenocarcinomas were observed in the group treated with Fe₂O₃ + BaP. One was located in trachea and one in the lungs. Sarcomas were found in two hamsters treated with oil fly ash + BaP. The location was trachea and the lungs. Also in one animal treated with only BaP a tracheal sarcoma was observed. Of the four hamsters with malignant lymphoma two hamsters had lymphomas located in the lungs.

^bIn one of the hamsters the same cancer was located both in larynx and trachea.

block the airways and thus kill the hamster. Such papillomas we designated fatal benign tumors. An illustrative example of this type of papilloma is shown in Figure 3.

In hamsters with malignant tumors, squamous cell carcinoma was the most frequent tumor. This type of carcinoma occurred in all regions of the respiratory tract examined. In some of the hamsters with squamous cell carcinoma, the same tumor could grow from one region into another, e.g., from larynx to trachea. Squamous cell carcinoma was most frequent in hamsters treated with oil fly ash coated with BaP. A typical example of squamous cell carcinoma is shown in Figure 4. The most frequent malignant tumors observed in the lungs were the squamous cell carcinoma and the adenosquamous carcinomas. As for squamous cell carcinoma, the highest

incidence of adenosquamous carcinoma was found in hamsters treated with oil fly ash coated with BaP. Other malignant tumors observed at a low frequency were adenocarcinomas and sarcomas.

Tumors Observed in Other Regions Than the Respiratory Tract. In hamsters instilled with suspensions containing BaP, there was a high incidence of squamous papilloma of the stomach (Table 6). The frequency of gastric papilloma in this group was significantly higher than that in hamsters treated with suspensions without BaP. In two hamsters instilled with suspensions containing BaP, squamous cell carcinoma was observed in the stomach. Malignant lymphoma was observed in four hamsters. In two of these animals, the malignant lymphoma spread to the lungs. In some hamsters, single observations of benign or malignant tumors of varying

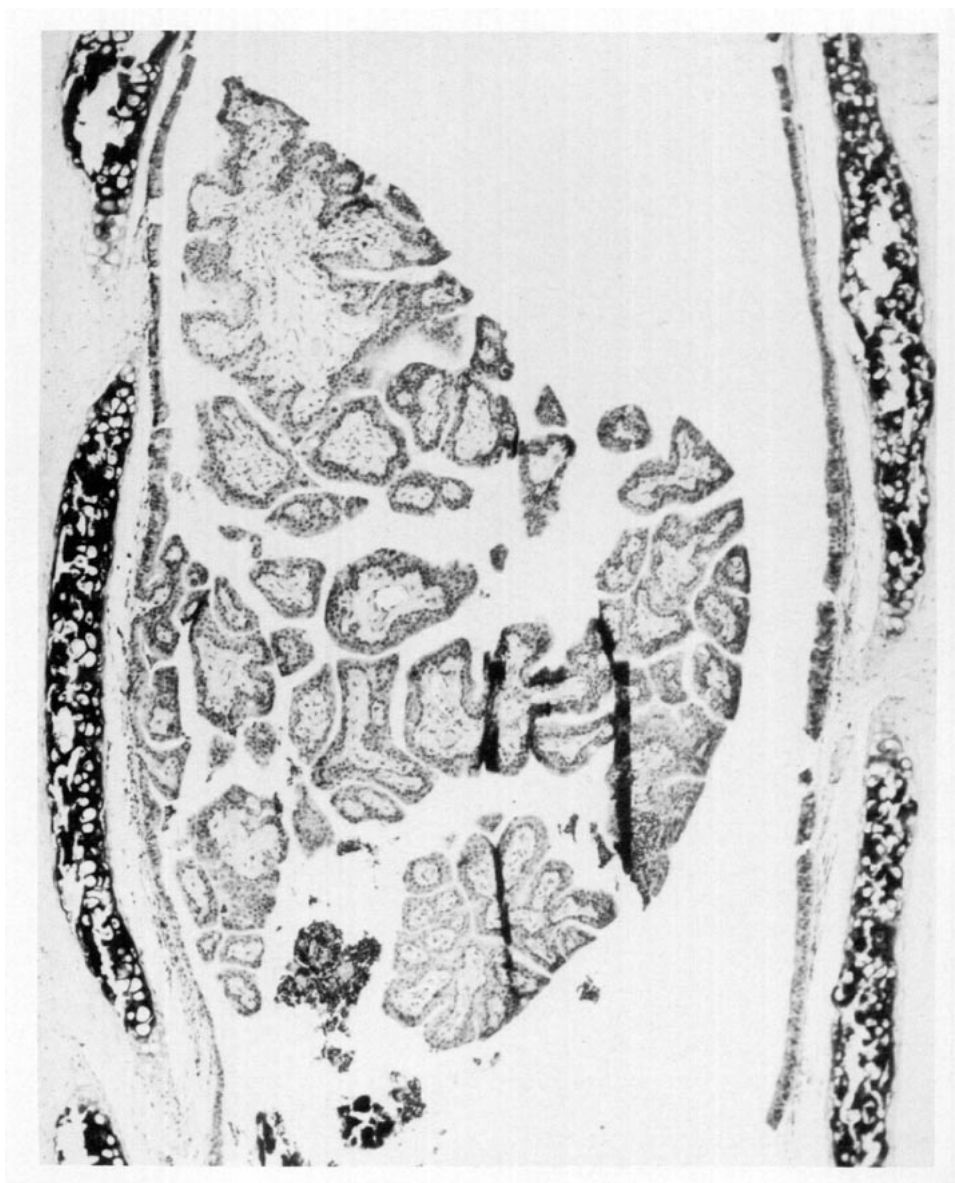


FIGURE 3. Large squamous cell papilloma in trachea (longitudinal section). H & E, $\times 90$.

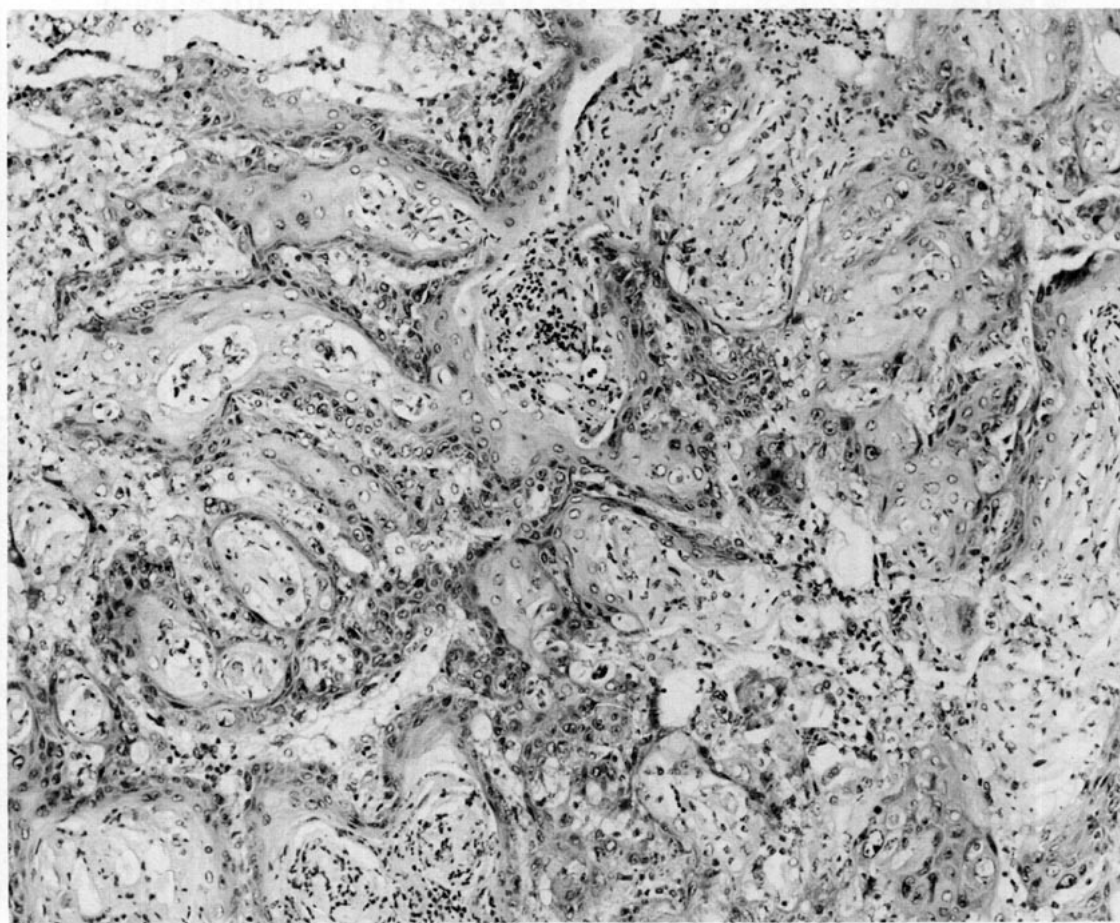


FIGURE 4. Squamous cell carcinoma in the lung. H & E, $\times 150$.

type were made in different regions. The variety in type of malignancy and the low frequency of these tumors made us exclude them from further discussion in this paper.

Probability to Die with Benign or Malignant Tumor or to Die from Tumor in the Respiratory Tract

Figure 5 shows the probability of death with tumor of any type in the respiratory pathways. In comparison among the various groups, the week when a tumor was detected in a hamster was considered. Since no tumor was detected in a living hamster, this week was always the week when the hamster died or was killed. In the testing, we have also considered the number of surviving hamsters in the week a tumor was detected in a dead hamster.

Low probability of death with a tumor was observed in hamsters treated with particle suspensions without BaP. Among these animals, hamsters treated with oil fly ash (high dose) had the highest probability of death (0.21). In hamsters treated with BaP or with BaP-

coated particles, animals treated with oil fly ash coated with BaP had the maximal probability (1.0) to die with a tumor 95 weeks after beginning the treatment. A probability of 1.0 was also reached for hamsters treated with ferric oxide coated with BaP or coal fly ash coated with BaP, but this probability occurred later (at week 99 and 113, respectively). Interestingly, hamsters treated with only BaP never reached a probability of death with a tumor higher than 0.58.

Table 6. Number of hamsters with squamous papilloma in the stomach.

Code	Treatment	N	Number with tumor
A	Vehicle	60	1
Ba	Coal fly ash (low dose)	58	1
Bb	Coal fly ash (high dose)	30	
Ca	Oil fly ash (high dose)	59	3
Cb	Oil fly ash (low dose)	29	
D	Ferric oxide, Fe_2O_3	58	
E	BaP	59	12
F	Coal fly ash + BaP	59	9
G	Oil fly ash + BaP	58	5
H	Fe_2O_3 + BaP	60	18

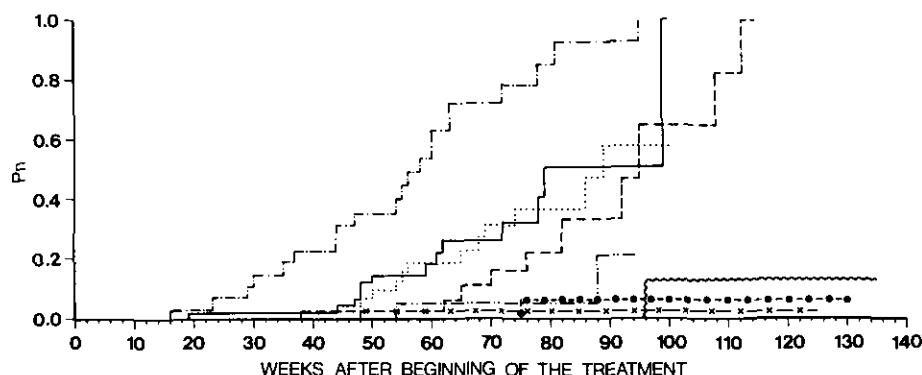


FIGURE 5. The probability (P_n) to die with a tumor in the respiratory tract in the controls and in the various treatment groups during 120 weeks after beginning of the treatment. Saline (x—x); coal fly ash (low dose) (~—~); oil fly ash (high dose) (-.-.-); Fe_2O_3 (●—●); BaP (.....); coal fly ash coated with BaP (——); oil fly ash coated with BaP (-.-.-); Fe_2O_3 coated with BaP (—)

The probability for a hamster to die with a malignant tumor is shown in Figure 6. Hamsters treated with oil fly ash coated with BaP and ferric oxide coated with BaP had the maximal probability to die with a malignant tumor in week 99 after the beginning of the treatment. Since all hamsters that were treated with ferric oxide coated with BaP and died in week 99 had malignant tumors, there was a rapid increase in probability. However, earlier, the hamsters treated with oil fly ash coated with BaP showed a higher probability of death with malignant tumor than any of the other groups. Up to 95 weeks after the beginning of the treatment, hamsters treated with coal fly ash coated with BaP had the lowest probability of death with malignant tumor, but then the probability did not differ from that of hamsters treated with only BaP. We examined the probability of a hamster to die from a tumor. Tumors that could be cause of death are all malignant tumors or fatal benign tumors. Like the malignant tumors, fatal benign tumors occurred only in hamsters treated with suspensions containing BaP. The highest probability of death from a tumor was found in hamsters treated with oil fly ash coated with BaP. The other groups of hamsters did not

significantly differ from each other concerning this probability.

Discussion

The purpose of the present investigation was to examine the carcinogenicity in the respiratory tract of coal fly ash, sampled in a manner to obtain a material with close similarity to emissions from a coal-fired power plant. In this study we have compared the carcinogenicity of coal fly ash with particulate emissions from an oil-fired power plant, oil fly ash, sampled in a similar way.

The oil fly ash was sampled before the electrostatic precipitator, since it was impossible, for practical reasons, to obtain the amount of material needed by sampling after the precipitator. For the sampling method used the concentration was too low. In Sweden, however, most oil-fired boilers are not equipped with electrostatic precipitators, and it has also been shown that large particles, not regarded as respirable, may deposit in the lung after inhalation (20). Thus, the use of these large oil fly ash particles in this carcinogenicity study may have relevance.

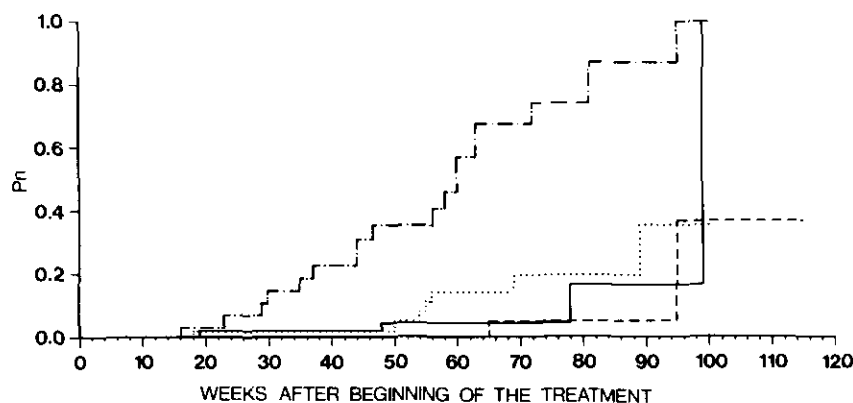


FIGURE 6. Probability (P_n) to die with malignant tumor in the respiratory tract in the controls and in the various treatment groups various weeks after beginning of the treatment. Symbols as in Fig. 5.

The fact that the elements found to be enriched on small coal fly ash particles (V, Cu, Zn, Ga, Se, Sr, Ba, and Pb) were also enriched on the impactor collected particles when compared with analyses of particles from the electrostatic precipitator indicates that a condensation of volatile elements onto the particles really occurred in the sampling probe used (2,3).

The filter fraction of the coal fly ash was omitted in the blending of the test material. This means that toxic elements, enriched on particles during condensation, will show lower concentrations in the test material than in the originally collected fly ash. The filter fraction constituted 13% by weight of the initially collected coal fly ash. The omission of this fraction resulted in a decrease of about 25% for the two elements, which showed highest enrichment on small particles (Zn and Se) when compared with the concentrations in the initially collected fly ash (2).

The coal and oil fly ashes were found to contain some water-soluble material (2). In the preparation of the BaP-coated fly ashes, the particles were suspended in water, which was then filtered off (see above). The BaP-coated particulate material was thereafter scraped off the filter and resuspended in the saline solution used for the instillations. The uncoated fly ash was suspended directly in the saline solution. This means that the suspensions of BaP-coated fly ash contained less water-soluble material than suspensions of uncoated fly ash.

The dose level chosen for uncoated material was 4.5 mg, of which 85 to 90% was delivered by instillation. The dose level for BaP-coated material was $4.5 + 4.5 = 9$ mg. However, since only about 50% of this material was delivered by instillation (Table 3), the doses administered are comparable.

We used intratracheal instillation in Syrian golden hamsters as an animal model. The Syrian golden hamster has not been reported to have spontaneous epithelial tumors in the respiratory tract (12). The material to be examined is directly delivered to the respiratory pathways. It is possible to induce a high incidence of tumors in the respiratory tract with particles coated with BaP. We appeared to have success in using this model, since we obtained tumors in hamsters treated with BaP-coated ferric oxide, but not in hamsters instilled with ferric oxide alone. The frequency of tumors we observed was low in comparison with that obtained by others (5,6). Interestingly, the hamsters treated with BaP alone showed a rather high incidence of tumors. This finding is at variance with results obtained in other studies (5,6), but is in agreement with the observations of Pershagen et al. (7). One explanation of this difference in tumor incidence could be different sensitivity to BaP in various strains of hamsters (21).

Fifteen weeks after beginning the instillations, i.e., when all hamsters had received their 15 instillations, a decrease in survival rate was observed in animals treated with oil fly ash or oil fly ash coated with BaP (Fig. 2). The survival rates were 69 and 59%, respectively, while the survival rate in the other groups was 81 to 95%. Although a fatal (malignant) tumor in ham-

sters treated with BaP-coated oil fly ash was observed already 16 weeks after beginning the treatment, an increased tumor incidence appears to be an unlikely explanation of this early decreased survival rate in hamsters treated with oil fly ash. The reason for this is the fact that a fatal tumor was never observed in hamsters treated with oil fly ash without BaP. We have previously reported a decreased phagocytosis of the alveolar macrophages in hamsters treated with oil fly ash compared with controls and with hamsters treated with coal fly ash (2). The decreased phagocytosis was also observed when the oil fly ash particles were removed. Therefore, the observed early decrease of survival rate could be due to a water-soluble toxic principle in the oil fly ash. This may be true for oil fly ash without BaP, but, as mentioned above, particles coated with BaP were washed during the coating procedure. Thus, it is unlikely that a water-soluble principle remained after this procedure. The early decrease in survival could hence have another explanation. For example, the particle size could be of importance. Recently, vanadium pentoxide and vanadium sulfate were reported to have cytotoxic effects on hamster tracheal epithelium (22). Oil fly ash showed similar effects. It is interesting to note that, as expected, the elemental analyses of the particulate material used in the present study showed that oil fly ash had a much higher percentage of vanadium than coal fly ash. The incidence of malignant tumors was probably important for the later decrease of survival rate in hamsters treated with BaP-coated oil fly ash.

As regards epithelial changes that could not be characterized as tumors, we found that hamsters treated with oil fly ash with or without BaP had more bronchiolar-alveolar hyperplasia than the control groups or groups treated with coal fly ash with or without BaP (Table 4). The most pronounced differences between the groups were observed in animals with bronchiolar-alveolar hyperplasia with high degrees of intensity. Marked squamous epithelial dysplasia could be considered as a form of cancer *in situ* [a lesion in which all or most of the epithelium shows the cellular features of carcinoma and with no invasion of the underlying stroma (23)]. We therefore found it of interest to examine any differences in squamous dysplasia between the various treatments. In the larynx and trachea, but not in the bronchi, hamsters treated with BaP-coated particles had a significantly higher frequency of squamous cell dysplasia than those instilled with uncoated particles (Table 4). There was no significant change in squamous dysplasia between any of the individual groups of hamsters. A similar result was obtained as regards squamous metaplasia (Table 4). However, only in trachea there was an increased frequency of squamous metaplasia in hamsters treated with BaP-coated particles.

Hamsters treated with oil fly ash coated with BaP had by far the highest tumor incidence. Twenty-one of 58 hamsters (36%) had tumors, and 18 of those had malignant tumors. Ten (17%) of the 59 hamsters treated with coal fly ash coated with BaP developed tumors. Only two animals had malignant tumors. In hamsters

treated with oil fly ash coated with BaP, malignant tumors occurred earlier than in hamsters with other treatments. The malignant tumors occurring were almost all of epithelial origin, e.g., squamous cell carcinoma or adenosquamous carcinoma. Hamsters treated with oil fly ash coated with BaP also had the highest probability to die with a tumor or to die with a malignant tumor (Figs. 5 and 6). This group also had a significantly higher probability to die from a tumor.

In hamsters instilled with BaP or particles coated with BaP, a high incidence of squamous papilloma was observed in the stomach (Table 6). Furthermore, two hamsters were found to have carcinoma of the stomach. These findings suggest that the gastrointestinal tract was exposed to high amounts of the instilled particulate material. The clearance of instilled particles through the GI was not determined. However, as previously mentioned, single instillations of BaP or BaP-coated particulate materials showed that the lung levels of BaP were approximately 100 to 1000 times higher than those in the GI. Since the tumor incidence was much higher in hamsters treated with BaP or particles coated with BaP than in the other groups of hamsters, it seems clearly demonstrated that BaP was important for the production of the stomach squamous papillomas and carcinomas. The increased stomach tumor incidence could probably not be explained by a high exposure of this tissue to BaP by means of a great clearance of BaP through the GI.

The tumor incidence in hamsters treated with suspensions of particles not coated with BaP was low. Furthermore, in these groups no malignant tumor was observed. The higher toxicity of the oil fly ash particles and the increased carcinogenicity of the same particles coated with BaP, as compared with the other particulate materials, are difficult to explain. Mutagenicity studies performed with *Salmonella* strains TA98 and TA100 showed a mutagenicity below the detection limit (2). In addition, the particulate material tested for mutagenicity was not directly comparable to that used in the cancer study, since it was from a different sampling line with a different sampling method (2).

Particle size could be of importance in the carcinogenicity of the oil fly ash particles. It has been shown that intratracheal instillation of insoluble particles coated with BaP will increase the retention of BaP in the respiratory tract and thereby increase the time during which the organic carcinogen is in contact with the mucosa of the respiratory tract (11,24,25). Our results are in agreement with these findings (Table 3). An increased lung retention of particle-associated BaP has also been demonstrated in inhalation studies of BaP adsorbed to $^{67}\text{Ga}_2\text{O}_3$ -particles (26) and BaP-associated with diesel exhaust particles (27). Particle size appears to be of great importance for the retention of particle-associated BaP and also for tumor induction (5,11). However, the rate by which BaP is eluted from the particles may be more important than the retention of the particles in the lung (28). We have shown an increased retention of BaP in the lung after instillation of

a single dose of BaP-coated oil fly ash particles. We have not measured the elution rate of BaP from these particles, but no data of our study suggest any increased elution rate of BaP from the oil fly ash particles. Therefore, the size of the fly ash particles appears to be of importance for the results obtained in the present study.

Hamsters treated with suspensions of oil fly ash had a high incidence of tissue lesions in the respiratory tract. Oil fly ash particles coated with BaP were also carcinogenic. The carcinogenic effect of BaP-coated oil fly ash could be characterized as a cocarcinogenic effect, since particles and BaP were administered together. The more cytotoxic oil fly ash might be acting as a promoter of tumor production by the carcinogen BaP. Such a promoter effect cannot be excluded, but has not been examined in the present study. It can be argued that the oil fly ash particles used in this study are so large that they could rarely reach the lung by inhalation (29,30). However, a recent inhalation study in humans (20) shows that particles of considerable size could reach the lung.

Coal fly ash did not appear to decrease the survival rate of the hamsters even when these particles were coated with BaP. In addition, hamsters treated with BaP-coated coal fly ash also had a low incidence of malignant tumors. The present study gives no indication that coal fly ash could create more serious health problems than oil fly ash.

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